

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**  
**REQUEST FOR FILING NATIONAL PHASE OF**  
**PCT APPLICATION UNDER 35 U.S.C. 371 AND 37 CFR 1.494 OR 1.495**

To: Hon. Commissioner of Patents  
 Washington, D.C. 20231



00909

TRANSMITTAL LETTER TO THE UNITED STATES  
 DESIGNATED/ELECTED OFFICE (DO/EO/US)

Atty Dkt: PM 275912 /PHM/Z 70365/UST  
M# /Client Ref.

From: Pillsbury Madison & Sutro LLP, IP Group:

Date: January 18, 2001

This is a **REQUEST** for **FILING** a PCT/USA National Phase Application based on:

|                              |                                   |   |
|------------------------------|-----------------------------------|---|
| 1. International Application | 2. International Filing Date      | 3. Earliest Priority Date Claimed                     |
| <u>PCT/GB99/02340</u>        | <u>20</u> <u>July</u> <u>1999</u> | <u>25</u> <u>July</u> <u>1998</u>                     |
| <u>1</u> country code        | Day MONTH Year                    | Day MONTH Year<br>(use item 2 if no earlier priority) |

4. Measured from the earliest priority date in item 3, this PCT/USA National Phase Application Request is being filed within:

(a) ☐ 20 months from above item 3 date (b) ☒ 30 months from above item 3 date,

(c) Therefore, the due date (unextendable) is January 25, 2001

Title of Invention GENETIC POLYMORPHISMS IN THE HUMAN NEUROKININ 1 RECEPTOR GENE AND THEIR USE IN DIAGNOSIS AND TREATMENT OF DISEASES

Inventor(s) SMITH, John Craig et al

Applicant herewith submits the following under 35 U.S.C. 371 to effect filing:

☒ Please immediately start national examination procedures (35 U.S.C. 371 (f)).

☒ **A copy of the International Application** as filed (35 U.S.C. 371(c)(2)) is transmitted herewith (file if in English but, if in foreign language, file only if not transmitted to PTO by the International Bureau) including:

- a. ☒ Request;  
 b. ☒ Abstract;  
 c. 33 pgs. Spec. and Claims;  
 d.        sheet(s) Drawing which are ☐ informal ☐ formal of size ☐ A4 ☐ 11"

☒ **A copy of the International Application has been transmitted by the International Bureau.**

**A translation of the International Application** into English (35 U.S.C. 374(c)(2))

- a. ☐ is transmitted herewith including: (1) ☐ Request; (2) ☐ Abstract;  
 (3)        pgs. Spec. and Claims;  
 (4)        sheet(s) Drawing which are:  
       ☐ informal ☐ formal of size ☐ A4 ☐ 11"
- b. ☐ is not required, as the application was filed in English.  
 c. ☐ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.  
 d. ☐ Translation verification attached (not required now).

11. ☒ **PLEASE AMEND** the specification before its first line by inserting as a separate paragraph:  
 a. ☒ --This application is the national phase of international application PCT/GB99/02340 filed July 20, 1999 which designated the U.S.--  
 b. ☐ --This application also claims the benefit of U.S. Provisional Application No. 60/\_\_\_\_, filed \_\_\_\_--
12. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., **before 18th month** from first priority date above in item 3, are transmitted herewith (file only if in **English**) including:
13. ☒ PCT Article 19 claim amendments (if any) have been transmitted by the International Bureau
14. ☐ Translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., of claim amendments made before 18th month, is attached (required by 20th month from the date in item 3 if box 4(a) above is X'd, or 30th month if box 4(b) is X'd, or else amendments will be considered **canceled**).
15. **A declaration of the inventor** (35 U.S.C. 371(c)(4))  
 a. ☒ is submitted herewith ☒ Original ☐ Facsimile/Copy  
 b. ☐ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.
16. **An International Search Report (ISR):**  
 a. Was prepared by ☒ European Patent Office ☐ Japanese Patent Office ☐ Other  
 b. ☒ has been transmitted by the international Bureau to PTO.  
 c. ☒ copy herewith (3 pg(s).) ☒ plus Annex of family members (2 pg(s).).
17. **International Preliminary Examination Report (IPER):**  
 a. ☒ has been transmitted (if this letter is filed after 28 months from date in item 3) in English by the International Bureau with Annexes (if any) in original language.  
 b. ☒ copy herewith in **English**.  
 c.1 ☐ IPER Annex(es) in original language ("Annexes" are amendments made to claims/spec/drawings during **Examination**) including attached amended:  
 c.2 ☐ Specification/claim pages #\_\_\_\_ claims #\_\_\_\_  
 Dwg Sheets #\_\_\_\_  
 d. ☐ Translation of Annex(es) to IPER (**required by 30<sup>th</sup> month due date, or else annexed amendments will be considered canceled**).
18. **Information Disclosure Statement** including:  
 a. ☒ Attached Form PTO-1449 listing documents  
 b. ☐ Attached copies of documents listed on Form PTO-1449  
 c. ☒ A concise explanation of relevance of ISR references is given in the ISR.
19. ☒ **Assignment** document and Cover Sheet for recording are attached. Please mail the recorded assignment document back to the person whose signature, name and address appear at the end of this letter.
20. ☐ Copy of Power to IA agent.
21. ☐ **Drawings** (complete only if 8d or 10a(4) not completed): \_\_\_\_ sheet(s) per set: ☐ 1 set informal; ☐ Formal of size ☐ A4 ☐ 11"
22. Small Entity Status ☐ is **Not** claimed ☐ is claimed (**pre-filing confirmation required**)  
 22(a) \_\_\_\_ (No.) Small Entity Statement(s) enclosed (since 9/8/00 Small Entity Statements(s) not essential to make claim)
23. **Priority** is hereby claimed under 35 U.S.C. 119/365 based on the priority claim and the certified copy, both filed in the International Application during the international stage based on the filing in (country) **GREAT BRITAIN** of:
- |     | Application No. | Filing Date   |     | Application No. | Filing Date     |
|-----|-----------------|---------------|-----|-----------------|-----------------|
| (1) | 9816192.0       | July 25, 1998 | (2) | 9818280.1       | August 22, 1998 |
| (3) | _____           | _____         | (4) | _____           | _____           |
| (5) | _____           | _____         | (6) | _____           | _____           |
- a. ☒ See Form PCT/IB/304 sent to US/DO with copy of priority documents. If copy has not been received, please proceed promptly to obtain same from the IB.  
 b. ☐ Copy of Form PCT/IB/304 attached.

24. Attached: 3 Pages of Sequence Listing and 2 pages of Form PCT/IB/306

25. **Preliminary Amendment:**25.5 Per Item 17.c2, cancel original pages # \_\_\_\_\_, claims # \_\_\_\_\_, Drawing Sheets # \_\_\_\_\_26. **Calculation of the U.S. National Fee (35 U.S.C. 371 (c)(1)) and other fees is as follows:**Based on amended claim(s) per above item(s) ☐ 12, ☐ 14, ☐ 17, ☐ 25, ☐ 25.5 (hite)

|  |    |            |   |                 |   |       |         |
|--|----|------------|---|-----------------|---|-------|---------|
| Total Effective Claims   | 22 | minus 20 = | 2 | x \$18/\$9      | = | \$36  | 966/967 |
| Independent Claims   | 10 | minus 3 =  | 7 | x \$80/\$40     | = | \$560 | 964/965 |
| If any proper (ignore improper) Multiple Dependent claim is present, |    |            |   | add \$270/\$135 | = | +270  | 968/969 |

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)): →→ **BASIC FEE REQUIRED, NOW** →→→→A. If country code letters in item 1 are not "US", "BR", "BB", "TT", "MX", "IL", "NZ", "IN" or "ZA" ↓See item 16 re: ↓

|  |                  |         |
|--|------------------|---------|
| 1. Search Report was <u>not prepared by EPO or JPO</u> ----- | add \$1000/\$500 | 960/961 |
| 2. Search Report was prepared by EPO or JPO -----            | add \$860/\$430  | 970/971 |
|  | <u>+860</u>      |         |

**SKIP B, C, D AND E UNLESS country code letters in item 1 are "US", "BR", "BB", "TT", "MX", "IL", "NZ", "IN" or "ZA"** ↓

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|--|-----------------|----|---------|
| <input type="checkbox"/> B. If <u>USPTO</u> did not issue <u>both</u> International Search Report (ISR) <u>and</u> (if box 4(b) above is X'd) the International Examination Report (IPER), -----                           | add \$970/\$485 | +0 | 960/961 |
| <input type="checkbox"/> C. If <u>USPTO</u> issued ISR but not IPER (or box 4(a) above is X'd), -----  | add \$710/\$355 | +0 | 958/959 |
| <input type="checkbox"/> D. If <u>USPTO</u> issued IPER but IPER Sec. V boxes <u>not all</u> 3 YES, -----  | add \$690/\$345 | +0 | 956/957 |
| <input type="checkbox"/> E. If international preliminary examination fee was paid to <u>USPTO</u> <u>and</u> Rules 492(a)(4) and 496(b) <u>satisfied</u> (IPER Sec. V <u>all</u> 3 boxes YES for <u>all</u> claims), ----- | add \$100/\$50  | +0 | 962/963 |

27. **SUBTOTAL =** \$172628. If Assignment box 19 above is X'd, add Assignment Recording fee of ----\$40 +40 (581)29. Attached is a check to cover the ----- **TOTAL FEES** \$1766

Our Deposit Account No. 03-3975

Our Order No. 9901 | 275912  
C# M#

00909

**CHARGE STATEMENT:** The Commissioner is hereby authorized to charge any fee specifically authorized hereafter, or any missing or insufficient fee(s) filed, or asserted to be filed, or which should have been filed herewith or concerning any paper filed hereafter, and which may be required under Rules 16-18 and 492 (missing or insufficient fee only) now or hereafter relative to this application and the resulting Official document under Rule 20, or credit any overpayment, to our Account/Order Nos. shown above for which purpose a duplicate copy of this sheet is attached.

This CHARGE STATEMENT does not authorize charge of the issue fee until/unless an issue fee transmittal form is filedPillsbury Winthrop LLP  
Intellectual Property GroupBy Atty: Donald J. Bird Reg. No. 25323Sig: [Signature] Fax: (202) 822-0944  
Tel: (202) 861-3027

Atty/Sec: DJB/mhn

**NOTE:** File in duplicate with 2 postcard receipts (PAT-103) & attachments.

# APPLICATION UNDER UNITED STATES PATENT LAWS

Atty. Dkt. No. PM 275912/PHM/Z70365/UST  
(M#)

Invention: GENETIC POLYMORPHISMS IN THE HUMAN NEUROKININ 1 RECEPTOR GENE AND THEIR USE IN DIAGNOSIS AND TREATMENT OF DISEASES

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## This is a:

- ☐ Provisional Application
- ☐ Regular Utility Application
- ☐ Continuing Application
  - ☒ The contents of the parent are incorporated by reference
- ☒ PCT National Phase Application
- ☐ Design Application
- ☐ Reissue Application
- ☐ Plant Application
- ☐ Substitute Specification
  - Sub. Spec. Filed \_\_\_\_\_
  - in App. No. \_\_\_\_\_ / \_\_\_\_\_
- ☐ Marked up Specification re
  - Sub. Spec. filed \_\_\_\_\_
  - In App. No. \_\_\_\_\_ / \_\_\_\_\_

## SPECIFICATION

GENETIC POLYMORPHISMS IN THE HUMAN NEUROKININ 1 RECEPTOR GENE AND THEIR USES IN DIAGNOSIS AND TREATMENT OF DISEASES

This invention relates to polymorphisms in the human NK1R gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic variation in the NK1R gene and to the use of said polymorphism in the diagnosis and treatment of NK1R ligand mediated diseases, such as asthma.

The reader is referred to the following publications for background information: Primary structure and gene organization of human substance P and neuromedin K receptors, K Takahashi *et al* Eur J Biochem **204**, 1025-1033 (1992); Differential activation of intracellular effector by two isoforms of the human neurokinin-1 receptor, TM Fong *et al* Molecular Pharmacology **41**, 24-30 (1992); Human Substance P receptor (NK-1): organisation of the gene, chromosome localization, and functional expression of cDNA clones, Gerard *et al* Biochemistry **30**, 10640-10646 (1991); Isolation and characterization of the human lung NK-1 receptor cDNA, Hopkins *et al*, Biochem Biophys Res Commun **180**, 110-1117 (1991); Mutational analysis of neurokinin receptor function. TM Fong *et al* Can J Physiol Pharmacol **73**, 860-865 (1995); Structure and function of G protein-coupled receptors, CD Strader *et al* Annual Reviews Biochemistry **63**, 101-132 (1994); The evolution and structure of aminergic G protein-coupled receptors, D Donnelly *et al* Receptors and Channels **2**, 61-78 (1994).

20 NK1R polypeptide is known to exist in 2 isoforms, which are possibly alternatively spliced variants of a single NK1R gene, see TM Fong *et al* Molecular Pharmacology **41**, 24-30 (1992). A cDNA encoding NK1R has been published in International patent application WO 92/16547, Children's Medical Center; and in European patent application EP 510 878, Merck.

The complete genomic sequence of NK1R is not presently known but regions thereof containing exons 1, 3 & 5 have been published by EMBL as follows: Exon 1, Accession Number X 65177, 2472 bp; Exon 3, Accession Number X65179, 373 bp; Exon 5, Accession Number X65181, 3929 bp. Apart from Accession Number X 65177, all positions herein relate to the positions indicated therein unless stated otherwise or apparent from the context.

The inventors have discovered that part of the sequence presented in EMBL Accession

30 Number X65177 is incorrect. Sequencing of genomic PCR products by the present inventors, has shown that the nucleotide sequence from positions 262 - 758 of EMBL Accession Number X65177 is incorrect and a Blast search of the data bases has shown that this

erroneous sequence actually corresponds to positions 1231-1729 of EMBL Accession Number U37688 which encodes a gene similar to the human c-myc proto-oncogene. None of the specific polymorphisms identified herein however, fall within this erroneous sequence.

A sequence containing the promoter region of the human NK1R gene has been published in "Structure, expression and second messenger-mediated regulation of the human and rat substance P receptors and their genes" JE Krause et al Regulatory Peptides 46, 59-66 (1993), (see Figure 2 - Conservation of the human and rat Substance P receptor gene putative promoter regions). The present inventors have confirmed that this sequence is correct. No accession number has been assigned to this published sequence. In view of the sequence error in EMBL X 65177, the corrected sequence is included herein as SEQ ID No. 1. This sequence has been used as the reference sequence for locating the position of the novel promoter and exon 1 (+ intron junction region) polymorphic variants of the NK1R gene identified herein.

With respect to SEQ ID No.1, nucleotides 1 - 261 correspond to sequences 1 - 261 in X 65177, sequence 262-833 replaces sequence 262-758 of X 65177 (resulting in an addition of 75 nucleotides to the complete sequence length) and sequence 834 - 2547 corresponds to sequence 759- 2472 of X 65177. Exon 1 ATG starts at position 2029 and ends at position 2417.

One approach is to use knowledge of polymorphisms to help identify patients most suited to therapy with particular pharmaceutical agents (this is often termed "pharmacogenetics"). Pharmacogenetics can also be used in pharmaceutical research to assist the drug selection process. Polymorphisms are used in mapping the human genome and to elucidate the genetic component of diseases. The reader is directed to the following references for background details on pharmacogenetics and other uses of polymorphism detection: Linder *et al.* (1997), Clinical Chemistry, 43, 254; Marshall (1997), Nature Biotechnology, 15, 1249; International Patent Application WO 97/40462, Spectra Biomedical; and Schafer *et al.* (1998), Nature Biotechnology, 16, 33.

A haplotype is a set of alleles found at linked polymorphic sites (such as within a gene) on a single (paternal or maternal) chromosome. If recombination within the gene is random, there may be as many as  $2^n$  haplotypes, where 2 is the number of alleles at each SNP and n is the number of SNPs. One approach to identifying mutations or polymorphisms which are correlated with clinical response is to carry out an association study using all the haplotypes

that can be identified in the population of interest. The frequency of each haplotype is limited by the frequency of its rarest allele, so that SNPs with low frequency alleles are particularly useful as markers of low frequency haplotypes. As particular mutations or polymorphisms associated with certain clinical features, such as adverse or abnormal events, are likely to be of low frequency within the population, low frequency SNPs may be particularly useful in identifying these mutations (for examples see: Linkage disequilibrium at the cystathionine beta synthase (CBS) locus and the association between genetic variation at the CBS locus and plasma levels of homocysteine. *Ann Hum Genet* (1998) 62:481-90, De Stefano V, Dekou V, Nicaud V, Chasse JF, London J, Stansbie D, Humphries SE, and Gudnason V; and Variation at the von willebrand factor (vWF) gene locus is associated with plasma vWF:Ag levels: identification of three novel single nucleotide polymorphisms in the vWF gene promoter. *Blood* (1999) 93:4277-83, Keightley AM, Lam YM, Brady JN, Cameron CL, Lillicrap D).

Clinical trials have shown that patient response to treatment with pharmaceuticals is often heterogeneous. Thus there is a need for improved approaches to pharmaceutical agent design and therapy.

Point mutations in polypeptides will be referred to as follows: natural amino acid (using 1 or 3 letter nomenclature), position, new amino acid. For (a hypothetical) example, "D25K" or "Asp25Lys" means that at position 25 an aspartic acid (D) has been changed to lysine (K). Multiple mutations in one polypeptide will be shown between square brackets with individual mutations separated by commas.

The present invention is based on the discovery of single nucleotide polymorphisms (SNPs) in the NK1R gene. As defined herein, the NK1R gene includes exon coding sequence, intron sequences intervening the exon sequences and, 3' and 5' untranslated region (3' UTR and 5' UTR) sequences, including the promoter element of the NK1R gene.

According to one aspect of the present invention there is provided a method for the diagnosis of a single nucleotide polymorphism in NK1R in a human, which method comprises determining the sequence of the nucleic acid of the human at one or more of positions: 2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177; 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181; and determining the status of the human by reference to polymorphism in the NK1R gene.

According to another aspect of the present invention there is provided a method for diagnosis of a single nucleotide polymorphism in NK1R in a human, which method comprises determining the sequence of the nucleic acid of the human at one or more positions:

- 2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;
- 5 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
- 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
- 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;
- 461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
- 495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
- 10 600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
- 809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
- and determining the status of the human by reference to polymorphism in NK1R or its 3' untranslated region.

According to another aspect of the present invention there is provided a method for 15 diagnosis of one or more single nucleotide polymorphism(s) in NK1R gene in a human, which method comprises determining the sequence of the nucleic acid of the human at one or more positions:

- 2361 in exon 1 as defined by the position in SEQ ID No. 1;
- 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
- 20 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
- 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;
- 461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
- 495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
- 600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
- 25 809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
- 1371 in the promoter element as defined by the position in SEQ ID No. 1;
- and determining the status of the human by reference to polymorphism in NK1R.

The term human includes both a human having or suspected of having a NK1R ligand mediated disease and an asymptomatic human who may be tested for predisposition or

- 30 susceptibility to such disease. At each position the human may be homozygous for an allele or the human may be a heterozygote.



In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 2361 in exon 1 is presence of C and/or T.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 271 near exon 3 is presence of G and/or T.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 272 near exon 3 is presence of A and/or a single base deletion at this position.

10 In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 245 in exon 5 is presence of C and/or a single base deletion at this position. This results in premature termination and loss of C-terminal 26 amino acids (see Example 1 below). Testing for the presence of this polymorphism is especially preferred because, without wishing to be bound  
15 by theoretical considerations, of its association with a significant loss of amino acids.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 461 in the 3'UTR is presence of G and/or C.

In another embodiment of the invention preferably the method for diagnosis described  
20 herein is one in which the single nucleotide polymorphism at position 495 in the 3'UTR is the presence of T and/or a single base insertion of A at this position.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 600 in the 3'UTR is presence of A and/or G.

25 In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 809 in the 3'UTR is presence of C and/or T.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 1371 in the promoter  
30 element is presence of A and/or G.

When considering positional relationships in the presence of a single base deletion or insertion the introduction of an appropriate gap is required in accordance with established techniques.

In another aspect of the invention we provide a method for the diagnosis of NK1R  
5 ligand-mediated disease, which method comprises:

- i) obtaining sample nucleic acid from an individual,  
detecting the presence or absence of a variant nucleotide at one or more of positions:  
2361 in exon 1 as defined by the position in SEQ ID No. 1;  
271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;  
10 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;  
245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;  
461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;  
495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;  
600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;  
15 809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;  
1371 in the promoter element as defined by the position in SEQ ID No. 1;  
and determining the status of the human by reference to polymorphism in NK1R.

The method for diagnosis is preferably one in which the sequence is determined by a  
method selected from allele specific amplification (i.e. ARMS<sup>TM</sup>-allele specific amplification;  
20 ARMS referring to amplification refractory mutation system), allele specific hybridisation  
(ASH), oligonucleotide ligation assay (OLA) and restriction fragment length polymorphism  
(RFLP).

The status of the human may be determined by reference to allelic variation at any one,  
two, three, four, five, six, seven, eight or all nine positions. The status of the human may also  
25 be determined by one or more of the specific polymorphisms identified herein in combination  
with one or more other SNP's.

NK1 antagonists have been explored by Glaxo, Pfizer, Merck, Parke-Davis, Lilly, RPR,  
and Sanofi, primarily for CNS indications. In a recent clinical trial it was reported that a  
single dose of Pfizer CP-122,721 inhibits emesis associated with chemotherapy and was well  
30 tolerated with no adverse effects (Kris *et al*, JNCI, 89, 817, 1997). Most recently Merck has  
announced positive Phase II studies with a NK1 antagonist in depression/anxiety.

It is believed that a dual NK1/ NK2 receptor antagonist will have some clinical utility, particularly for asthma. As compared with conventional therapies, it is expected that a dual NK1/NK2 receptor antagonist will better control airways hyper-responsiveness and neurogenic inflammation (extravasation and hypersecretion), both of which are characteristic manifestations of asthma. This multifaceted approach improves upon other therapies that are designed to treat only a single clinical manifestation of this disease. Other therapeutic opportunities for NK1/NK2 antagonist exist in pain, migraine, anxiety, depression, urinary incontinence, and inflammatory bowel disease.

Four companies have published on mixed NK1/NK2 receptor antagonists. Two of the compounds are peptides: FK-224 (Fujisawa) and S16474 (Servier). The other two, MDL-105,212 (Marion Merrell Dow) and a recent compound from Merck, are structurally related to the selective NK2 antagonist, SR48968 (Sanofi). Neurokinin receptor antagonists have been reviewed by C J Swain (1996) in *Exp. Opin. Ther. Patents*, 6, 367-378; and by Elliot & Seward (1997) in *Exp. Opin. Ther. Patents*, 7, 43-54.

The test sample of nucleic acid is conveniently a sample of blood, bronchoalveolar lavage fluid, sputum, urine or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique e.g. PCR, before analysis of allelic variation.

It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and optionally a signal generation system. Table 1 lists a number of mutation detection techniques, some based on the polymerase chain reaction (PCR). These may be used in combination with a number of signal generation systems, a selection of which is listed in Table 2. Further amplification techniques are listed in Table 3. Many current methods for the detection of allelic variation are reviewed by Nollau *et al.*, *Clin. Chem.* 43, 1114-1120, 1997; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2<sup>nd</sup> Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

## Abbreviations:

|        |   |
|--------|---|
| ALEX™  | Amplification refractory mutation system linear extension |
| APEX   | Arrayed primer extension                                  |
| ARMS™  | Amplification refractory mutation system                  |
| b-DNA  | Branched DNA  |
| CMC    | Chemical mismatch cleavage                                |
| bp     | base pair   |
| COPS   | Competitive oligonucleotide priming system                |
| DGGE   | Denaturing gradient gel electrophoresis                   |
| FRET   | Fluorescence resonance energy transfer                    |
| LCR    | Ligase chain reaction                                     |
| MASDA  | Multiple allele specific diagnostic assay                 |
| MCP-1  | Monocyte chemoattractant protein 1                        |
| NASBA  | Nucleic acid sequence based amplification                 |
| NK     | Neurokinin  |
| NK1R   | Neurokinin 1 receptor                                     |
| NK2R   | Neurokinin 2 receptor                                     |
| OLA    | Oligonucleotide ligation assay                            |
| PCR    | Polymerase chain reaction                                 |
| PTT    | Protein truncation test                                   |
| RFLP   | Restriction fragment length polymorphism                  |
| SDA    | Strand displacement amplification                         |
| SERRS  | Surface enhanced raman resonance spectroscopy             |
| SNP    | Single nucleotide polymorphism                            |
| SSCP   | Single-strand conformation polymorphism analysis          |
| SSR    | Self sustained replication                                |
| TGGE   | Temperature gradient gel electrophoresis                  |
| 3' UTR | 3' untranslated region                                    |

**Table 1 - Mutation Detection Techniques****General:** DNA sequencing, Sequencing by hybridisation**Scanning:** PTT\*, SSCP, DGGE, TGGE, Cleavase, Heteroduplex analysis, CMC, Enzymatic mismatch cleavage

5 \* Note: not useful for detection of promoter polymorphisms.

**Hybridisation Based**

Solid phase hybridisation: Dot blots, MASDA, Reverse dot blots, Oligonucleotide arrays (DNA Chips)

Solution phase hybridisation: Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi *et al* (1996), Nature Biotechnology, 14, 303; WO 95/13399 (Public Health Inst., New York)**Extension Based:** ARMS™-allele specific amplification (as described in European patent No. EP-B-332435 and US patent No. 5,595,890), ALEX™ - European Patent No. EP 332435 B1 (Zeneca Limited), COPS - Gibbs *et al* (1989), Nucleic Acids Research, 17, 2347.15 **Incorporation Based:** Mini-sequencing, APEX**Restriction Enzyme Based:** RFLP, Restriction site generating PCR**Ligation Based:** OLA**Other:** Invader assay20 **Table 2 - Signal Generation or Detection Systems****Fluorescence:** FRET, Fluorescence quenching, Fluorescence polarisation - United Kingdom Patent No. 2228998 (Zeneca Limited)**Other:** Chemiluminescence, Electrochemiluminescence, Raman, Radioactivity, Colorimetric, Hybridisation protection assay, Mass spectrometry, SERRS - WO 97/05280 (University of Strathclyde).  
25**Table 3 - Further Amplification Methods**

SSR, NASBA, LCR, SDA, b-DNA

30 Preferred mutation detection techniques include ARMS™-allele specific amplification, ALEX™, COPS, Taqman, Molecular Beacons, RFLP, OLA, restriction site based PCR and FRET techniques.

Particularly preferred methods include ARMS™-allele specific amplification, OLA and RFLP based methods. ARMS™-allele specific amplification is an especially preferred method.

ARMS™-allele specific amplification (described in European patent No. EP-B-332435, US patent No. 5,595,890 and Newton et al. (Nucleic Acids Research, Vol. 17, p.2503; 1989)), relies on the complementarity of the 3' terminal nucleotide of the primer and its template. The 3' terminal nucleotide of the primer being either complementary or non-complementary to the specific mutation, allele or polymorphism to be detected. There is a selective advantage for primer extension from the primer whose 3' terminal nucleotide complements the base mutation, allele or polymorphism. Those primers which have a 3' terminal mismatch with the template sequence severely inhibit or prevent enzymatic primer extension. Polymerase chain reaction or unidirectional primer extension reactions therefore result in product amplification when the 3' terminal nucleotide of the primer complements that of the template, but not, or at least not efficiently, when the 3' terminal nucleotide does not complement that of the template.

By way of example, a suitable allele specific primer (ARMS primer) capable of detecting/diagnosing the 2361 "T" polymorphism in Exon 1 is:  
5'-GCAAGTTCACAACCTTCTT-3' (SEQ ID No. 2). The 3' terminal nucleotide complementing the "A" polymorphism on the anti-sense template strand facilitates efficient primer extension with the suitable enzyme (preferably one lacking 3'-5' exonuclease activity).

In a further aspect, the diagnostic methods of the invention are used to assess the efficacy of therapeutic compounds in the treatment of NK1R ligand mediated diseases, such as asthma.

The polymorphisms identified in the present invention that occur in intron regions or in the promoter region are not expected to alter the amino acid sequence of the NK1 receptor, but may affect the transcription and/or message stability of the sequences and thus affect the level of the receptors in cells.

Assays, for example reporter-based assays, may be devised to detect whether one or more of the above polymorphisms affect transcription levels and/or message stability.

Individuals who carry particular allelic variants of the NK1R gene may therefore exhibit differences in their ability to regulate protein biosynthesis under different physiological conditions and will display altered abilities to react to different diseases. In addition, differences in protein regulation arising as a result of allelic variation may have a direct effect

on the response of an individual to drug therapy. The diagnostic methods of the invention may be useful both to predict the clinical response to such agents and to determine therapeutic dose.

In a further aspect, the diagnostic methods of the invention, are used to assess the predisposition and/or susceptibility of an individual to diseases mediated by NK1R ligands. The present invention may be used to recognise individuals who are particularly at risk from developing these conditions.

In a further aspect, the diagnostic methods of the invention are used in the development of new drug therapies which selectively target one or more allelic variants of the NK1R gene.

10 Identification of a link between a particular allelic variant and predisposition to disease development or response to drug therapy may have a significant impact on the design of new drugs. Drugs may be designed to regulate the biological activity of variants implicated in the disease process whilst minimising effects on other variants.

In a further diagnostic aspect of the invention the presence or absence of variant nucleotides is detected by reference to the loss or gain of, optionally engineered, sites recognised by restriction enzymes. For example the polymorphism at position 271 and 272 in exon 3 can be detected by digestion with the restriction enzymes RsaI and Cac8I respectively. Engineered sites include those wherein the primer sequences employed to amplify the target sequence participates along with the nucleotide polymorphism to create a restriction site (see 20 for example, Example 2 section 2 on 809 polymorphism in the 3' UTR (SEQ ID No. 6)).

According to another aspect of the present invention there is provided a nucleic acid comprising any one of the following polymorphisms:

- the nucleic acid sequence of EMBL ACCESSION NO. X 65177 with T at position 2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;
- 25 the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with T at position 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
- the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with a single base deletion at position 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
- the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with a single base deletion at 30 position 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;
- or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms.

According to another aspect of the present invention there is provided a nucleic acid comprising any one of the following polymorphisms:

- the nucleic acid sequence of EMBL ACCESSION NO. X 65177 with T at position 2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;
- 5 the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with T at position 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
- the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with a single base deletion at position 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
- the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with a single base deletion at
- 10 position 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;
- the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with C at position 461 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;
- the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with A inserted at position 495 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;
- 15 the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with G at position 600 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;
- the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with T at position 809 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;
- or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at
- 20 least one of the polymorphisms.

According to another aspect of the present invention there is provided a nucleic acid comprising any one of the following polymorphism containing sequences:

- the nucleic acid sequence of SEQ ID No. 1 with T at position 2361 in exon 1 as defined by the position in SEQ ID No. 1; the nucleic acid sequence of EMBL ACCESSION NO. X 65179
- 25 with T at position 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with a single base deletion at position 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with a single base deletion at position 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X
- 30 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with C at position 461 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;



- the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with A inserted at position 495 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with G at position 600 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with T at position 809 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of SEQ ID No. 1 with G at position 1371 in the promoter element as defined by the position in SEQ ID No. 1; or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms.
- 10 Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases.

The invention further provides nucleotide primers which can detect the polymorphisms of the invention.

- According to another aspect of the present invention there is provided an allele specific 15 primer capable of detecting a NK1R gene polymorphism of the invention.

- An allele specific primer is used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as used for ARMST<sup>TM</sup> assays. The allele specific primer is preferably 17- 50 nucleotides, more 20 preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

- An allele specific primer preferably corresponds exactly with the allele to be detected but derivatives thereof are also contemplated wherein about 6-8 of the nucleotides at the 3' terminus correspond with the allele to be detected and wherein up to 10, such as up to 8, 6, 4, 2, or 1 of the remaining nucleotides may be varied without significantly affecting the 25 properties of the primer. Often the nucleotide at the -2 and/or -3 position (relative to the 3' terminus) is mismatched in order to optimise differential primer binding and preferential extension from the correct allele discriminatory primer only.

- Primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for 30 Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1<sup>st</sup> Edition. If required the primer(s) may be labelled to facilitate detection.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a NK1R gene polymorphism of the invention.

The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

5 The design of such probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as up to 50 bases, up to 40 bases, more conveniently up to 30 bases in length, such as for example 8-25 or 8-15 bases in length. In general such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection, such as in Molecular Beacons.

According to another aspect of the present invention there is provided a diagnostic kit comprising one or more diagnostic probe(s) of the invention and/or diagnostic primer(s),  
15 particularly an allele-specific oligonucleotide primer, of the invention.

The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and polymerase(s) such as thermostable polymerases, for example taq polymerase. Such kits may also comprise companion/constant primers and/or control primers or probes. A  
20 companion/constant primer is one that is part of the pair of primers used to perform PCR. Such primer usually complements the template strand precisely.

In another aspect of the invention, the single nucleotide polymorphisms of this invention may be used as genetic markers in linkage studies. This particularly applies to the polymorphism in exon 1 (position 2361 in SEQ ID No. 1) because of its relatively high  
25 frequency (see below). Further preferred polymorphisms of high frequency are at positions 461 and 809 in the 3'UTR (see example 2 below). Those polymorphisms that occur relatively infrequently are useful as markers of low frequency haplotypes.

According to another aspect of the present invention there is provided an allelic variant of human NK1R polypeptide having a C-terminal deletion of 26 amino acids.

30 According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a NK1R ligand antagonist drug in which the method comprises:

- i) diagnosis of a single nucleotide polymorphism in NK1R gene in the human, which diagnosis comprises determining the sequence of the nucleic acid at one or more of positions:
    - 2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;
    - 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
    - 5 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
    - 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;
 and determining the status of the human by reference to polymorphism in the NK1R gene; and
  - ii) administering an effective amount of a NK1R ligand antagonist.
- 10 According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a NK1R ligand antagonist drug in which the method comprises:
- (i) diagnosis of a single nucleotide polymorphism in the NK1R gene in the human, which diagnosis comprises determining the sequence of nucleic acid at one or more of positions:
    - 15 2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;
    - 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
    - 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
    - 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;
    - 461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
    - 20 495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
    - 600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
    - 809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
 and determining the status of the human by reference to polymorphism in the NK1R gene; and
  - 25 (ii) administering an effective amount of a NK1R ligand antagonist.

According to another aspect of the present invention there is provided a method of treating a human in need of treatment with an NK1R ligand antagonist drug in which the method comprises:

- (i) diagnosis of a single nucleotide polymorphism in the NK1R gene in the human, which
- 30 diagnosis comprises determining the sequence of nucleic acid at one or more of positions:
  - 2361 in exon 1 as defined by the position SEQ ID No. 1;
  - 1371 in the promoter element as defined by the position in SEQ ID No. 1;

- 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;  
 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;  
 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;  
 461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;  
 5 495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;  
 600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;  
 809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;  
 and determining the status of the human by reference to polymorphism in the NK1R gene;  
 and
- 10 (ii) administering an effective amount of a NK1R ligand antagonist.

Preferably determination of the status of the human is clinically useful. Examples of clinical usefulness include deciding which antagonist drug of drugs to administer and/or in deciding on the effective amount of the drug or drugs. The NK1R ligand antagonist may optionally also have activity at the NK2R.

- 15 According to another aspect of the present invention there is provided use of an NK1R ligand antagonist drug in preparation of a medicament for treating a NK1R ligand mediated disease in a human diagnosed as having a single nucleotide polymorphism at one or more of positions:

2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;

- 20 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;  
 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;  
 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181.

- According to another aspect of the present invention there is provided use of an NK1R ligand antagonist drug in preparation of a medicament for treating a NK1R ligand mediated  
 25 disease in a human diagnosed as having a single nucleotide polymorphism at one or more of positions:

2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;

- 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;  
 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;  
 30 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;  
 461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;  
 495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181.

According to another aspect of the present invention there is provided use of an NK1R ligand antagonist drug in preparation of a medicament for treating a NK1R ligand mediated disease in a human diagnosed as having a single nucleotide polymorphism at one or more of positions:

2361 in exon 1 as defined by the position in SEQ ID No. 1;

1371 in the promoter element as defined by the position in SEQ ID No. 1;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

10 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

15 809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181.

According to another aspect of the present invention there is provided a pharmaceutical pack comprising an NK1R antagonist drug and instructions for administration of the drug to humans diagnostically tested for a single nucleotide polymorphism at one or more of positions:

20 2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181.

According to another aspect of the present invention there is provided a pharmaceutical pack comprising an NK1R antagonist drug and instructions for administration of the drug to humans diagnostically tested for a single nucleotide polymorphism at one or more positions:

2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

30 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;  
809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181.

- According to another aspect of the present invention there is provided a pharmaceutical pack comprising an NK1R antagonist drug and instructions for administration of the drug to 5 humans diagnostically tested for a single nucleotide polymorphism at one or more positions: 2361 in exon 1 as defined by the position in SEQ ID No. 1; 1371 in the promoter element as defined by the position in SEQ ID No. 1; 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
- 10 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181; 461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181; 495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181; 600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181; 809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181.
- 15 Testing for the presence of the polymorphism in exon 5 is especially preferred because, without wishing to be bound by theoretical considerations, of its resulting in a significant amino acid change in NK1R polypeptide (as explained herein).
- The nucleic acid sequences of the invention, particularly those relating to and identifying the single nucleotide polymorphisms identified herein represent a valuable information source 20 with which to identify further sequences of similar identity and characterise individuals in terms of, for example, their identity, haplotype and other sub-groupings, such as susceptibility to treatment with particular drugs. These approaches are most easily facilitated by storing the sequence information in a computer readable medium and then using the information in standard macromolecular structure programs or to search sequence databases using state of the 25 art searching tools such as GCG (Genetics Computer Group), BlastX BlastP, BlastN, FASTA (refer to Altschul et al. J. Mol. Biol. 215:403-410, 1990). Thus, the nucleic acid sequences of the invention are particularly useful as components in databases useful for sequence identity, genome mapping, pharmacogenetics and other search analyses. Generally, the sequence information relating to the nucleic acid sequences and polymorphisms of the invention may be 30 reduced to, converted into or stored in a tangible medium, such as a computer disk, preferably in a computer readable form. For example, chromatographic scan data or peak data, photographic scan or peak data, mass spectrographic data, sequence gel (or other) data.

The invention provides a computer readable medium having stored thereon one or more nucleic acid sequences of the invention. For example, a computer readable medium is provided comprising and having stored thereon a member selected from the group consisting of: a nucleic acid comprising the sequence of a nucleic acid of the invention, a nucleic acid consisting of a nucleic acid of the invention, a nucleic acid which comprises part of a nucleic acid of the invention, which part includes at least one of the polymorphisms of the invention, a set of nucleic acid sequences wherein the set includes at least one nucleic acid sequence of the invention, a data set comprising or consisting of a nucleic acid sequence of the invention or a part thereof comprising at least one of the polymorphisms identified herein. The computer readable medium can be any composition of matter used to store information or data, including, for example, floppy disks, tapes, chips, compact disks, digital disks, video disks, punch cards and hard drives.

In a particular embodiment of the invention there is provided a computer readable medium having stored thereon a member selected from the group consisting of: a nucleic acid comprising SEQ ID No. 1; a set of nucleic acids wherein at least one of said sequences comprises SEQ ID No. 1; a data set representing a nucleic acid sequence comprising SEQ ID No. 1; a nucleic acid consisting of SEQ ID No. 1; a set of nucleic acids wherein at least one of said sequences consists of the sequence of SEQ ID No. 1; a nucleic acid comprising any part (i.e. fragment of at least 20 bases) of a sequence selected from the group consisting of: SEQ ID No. 1, EMBL ACCESSION NO. X 65177, EMBL ACCESSION NO. X 65179, EMBL ACCESSION NO. X 65179 or EMBL ACCESSION NO. X 65181, which part includes at least one of the polymorphisms identified herein.

A computer based method is also provided for performing sequence identification, said method comprising the steps of providing a nucleic acid sequence comprising a polymorphism of the invention in a computer readable medium; and comparing said polymorphism containing nucleic acid sequence to at least one other nucleic acid or polypeptide sequence to identify identity (homology), i.e. screen for the presence of a polymorphism. Such a method is particularly useful in pharmacogenetic studies and in genome mapping studies.

In a particular embodiment of the invention there is provided a method for performing sequence identification, said method comprising the steps of providing a nucleic acid sequence comprising a sequence selected from the group consisting of:

- the nucleic acid sequence of SEQ ID No. 1 with T at position 2361 in exon 1 as defined by the position in SEQ ID No. 1; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with T at position 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with a single base deletion at position 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with a single base deletion at position 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with C at position 461 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the
- 10 nucleic acid sequence of EMBL ACCESSION NO. X 65181 with A inserted at position 495 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with G at position 600 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with T at position 809 in the 3'UTR as defined by the
- 15 position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of SEQ ID No. 1 with G at position 1371 in the promoter element as defined by the position in SEQ ID No. 1; or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms; and comparing said nucleic acid sequence to at least one other nucleic acid or polypeptide sequence to identify identity.
- 20 In another embodiment of the invention there is provided a method for performing sequence identification, said method comprising the steps of providing one or more of the following polymorphism containing nucleic acid sequences: the nucleic acid sequence of SEQ ID No. 1 with T at position 2361 in exon 1 as defined by the position in SEQ ID No. 1; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with T at position 271 near exon
- 25 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with a single base deletion at position 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with a single base deletion at position 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of
- 30 EMBL ACCESSION NO. X 65181 with C at position 461 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with A inserted at position 495 in the 3'UTR as defined by the



position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with G at position 600 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with T at position 809 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of SEQ ID No. 1 with G at position 1371 in the promoter element as defined by the position in SEQ ID No. 1; or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms, in a computer readable medium; and comparing said nucleic acid sequence to at least one other nucleic acid or polypeptide sequence to determine identity.

10 The invention will now be illustrated but not limited by reference to the following Examples. All temperatures are in degrees Celsius.

In the Examples below, unless otherwise stated, the following methodology and materials have been applied.

AMPLITAQ™, available from Perkin-Elmer Cetus, is used as the source of thermostable

15 DNA polymerase.

General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

Electropherograms were obtained in a standard manner: data was collected by ABI377 data collection software and the wave form generated by ABI Prism sequencing analysis (2.1.2).

## **EXAMPLES**

### **Example 1**

25 **Identification of Polymorphisms**

#### **1. Methods**

##### **DNA Preparation**

DNA was prepared from frozen blood samples collected in EDTA following protocol I (Molecular Cloning: A Laboratory Manual, p392, Sambrook, Fritsch and Maniatis, 2<sup>nd</sup> Edition, Cold Spring Harbor Press, 1989) with the following modifications. The thawed blood was diluted in an equal volume of standard saline citrate instead of phosphate buffered saline to remove lysed red blood cells. Samples were extracted with phenol, then

phenol/chloroform and then chloroform rather than with three phenol extractions. The DNA was dissolved in deionised water.

### **Template Preparation**

Templates were prepared by PCR using the oligonucleotide primers and annealing 5 temperatures set out below. The extension temperature was 72° and denaturation temperature 94°. Generally 50 ng of genomic DNA was used in each reaction and subjected to 35 cycles of PCR.

#### **Exon 1 SEQ ID No. 1 2547 bp**

10

| A). | Fragment  | Forward primer | Reverse Primer | Annealing Temp | Time |
|-----|-----------|----------------|----------------|----------------|------|
|     | 2000-2467 | 2000-2019      | 2448-2467      | 58°            | 60s  |

| B). | Fragment  | Forward Primer | Reverse Primer | Annealing Temp | Time |
|-----|-----------|----------------|----------------|----------------|------|
| 15  | 1168-1712 | 1168-1187      | 1693-1712      | 58°            | 60s  |

#### **Exon 3 Accession Number X65179 373 bp**

|    | Fragment | Forward Primer | Reverse Primer | Annealing Temp | Time |
|----|----------|----------------|----------------|----------------|------|
| 20 | 14-318   | 14-33          | 299-318        | 58°            | 60s  |

#### **Exon 5 Accession Number X65181 3929 bp**

|    | Fragment | Forward Primer | Reverse Primer | Annealing Temp | Time |
|----|----------|----------------|----------------|----------------|------|
| 25 | 18-417   | 18-38          | 398-417        | 58°            | 60s  |

For dye-primer sequencing these primers were modified to include M13 forward and reverse primer sequences (ABI protocol P/N 402114, Applied Biosystems) at the 5' end of the forward and reverse oligonucleotides respectively.

### 30 **Dye Primer Sequencing**

Dye-primer sequencing using M13 forward and reverse primers was as described in the ABI protocol P/N 402114 for the ABI Prism™ dye primer cycle sequencing core kit with

"AmpliTaQ FS"<sup>TM</sup> DNA polymerase, modified in that the annealing temperature was 45° and DMSO was added to the cycle sequencing mix to a final concentration of 5 %.

The extension reactions for each base were pooled, ethanol/sodium acetate precipitated, washed and resuspended in formamide loading buffer.

- 5 4.25 % Acrylamide gels were run on an automated sequencer (ABI 377, Applied Biosystems).

## 2. Results

### Exon 1 SEQ ID No. 1

10

A). Nucleotide 2361 C/T Phe (111) TTC/TTT

|                  |     |     |
|------------------|-----|-----|
| Allele frequency | TTC | 47% |
|                  | TTT | 53% |

- 15 To precisely identify the location of the 2361 polymorphism, its relative location within SEQ ID No. 1 is as follows:

TGCAAGTTCCACAACCTCTTCCCCATCGCCGCTGTCTTCGC (SEQ ID No. 3)

|      |      |      |
|------|------|------|
| 2341 | 2361 | 2381 |
|------|------|------|

- 20 B). Nucleotide 1371 A/G

|                                   |   |       |
|-----------------------------------|---|-------|
| Allele frequency (37 individuals) | A | 98.6% |
|                                   | G | 1.4 % |

- The polymorphism creates an recognition sequence (GGCCCC) for the restriction enzyme, Sau 96I (New England Biolabs). A PCR product ( position 1168-1712, 544 bp) containing the  
 25 wild type sequence (AGGCCC) will not be cleaved by Sau 96I (New England Biolabs).

Digestion of a heterozygote product (A/GGGCCC) will generate products of 203 bp, 341 bp and 540 bp. Digestion of a homozygous variant (GGCCCC) will generate products of 203 bp and 341 bp.

30

**Exon 3 Accession Number X 65179**

Flanking intron

**5 Nucleotide 271 G/T**

|                  |   |       |
|------------------|---|-------|
| Allele frequency | G | 98.5% |
|                  | T | 1.5%  |

**10 Nucleotide 272 ΔA (note "Δ" indicates deletion)**

|           |    |      |
|-----------|----|------|
| Frequency | ΔA | 4.5% |
|-----------|----|------|

To precisely identify the location of the 271 and 272 polymorphisms, their relative locations

**15 within the X65179 wild-type sequence is as follows:**

AAGTCTCTGCCAAGCGCAAGGTGAGCAGGGGACAGGCAGA (SEQ ID No. 4)

241 271 280

AAGTCTCTGCCAAGCGCAAGGTGAGCAGGGGACAGGCAGA (SEQ ID No. 5)

241 272 280

**20** The G271T polymorphism creates a RsaI (GTAC) restriction enzyme recognition site. A PCR product (304 bp) containing the wild type sequence will not be digested by RsaI. Digestion of a heterozyote product will give bands of 304bp, 257bp and 47 bp. Digestion of a homozygous variant product will generate bands of 257 bp, 47 bp.

The 272 ΔA polymorphism creates a Cac 8I (GCNNGC) restriction enzyme recognition **25** site. A PCR product (304 bp) containing the wild type sequence will not be digested by Cac 8I. Digestion of a heterozyote product will give bands of 304bp, 258bp and 46 bp. Digestion of a homozygous variant product will generate bands of 258 bp, 46 bp.

**Exon 5 Accession Number X 65181**Nucleotide 245  $\Delta$ C5 Frequency  $\Delta$ C 1.5%

This results in premature termination and loss of C-terminal 26 amino acids

... 379 380 381 382 379  
 10 S L D L S W T stop  
 TCC CTG GAC CTG.....  $\rightarrow$  ... TCC TGG ACC TGA

Unless otherwise indicated, all the allele frequencies were determined on the basis of analysis of 34 individuals.

15

**Example 2****Identification of Polymorphisms****1. Methods**

All PCR conditions and sequencing protocols are as described in Example 1. Allele frequencies were determined in a panel of 37 individuals.

**Template Preparation****3' UTR Accession Number X65181**

| Fragment    | Forward Primer | Reverse Primer | Annealing Temp | Time |
|-------------|----------------|----------------|----------------|------|
| 301-750     | 301-320        | 731-750        | 58°            | 60s  |
| 25 696-1144 | 696-715        | 1125-1144      | 58°            | 60s  |

**2. Results****3' UTR Accession Number: X65181**

|                |     |                  |       |
|----------------|-----|------------------|-------|
| Nucleotide 461 | G/C | Allele Frequency | G 72% |
| 30             |     |                  | C 28% |

-26-

This polymorphism can be detected by digestion with restriction enzyme Dde I

|          |               |             |                |
|----------|---------------|-------------|----------------|
| GTTAG... | DdeI negative | ...CTTAG... | Dde I positive |
| CAATC... |               | ...GAATC... |                |

|                  |             |                  |       |
|------------------|-------------|------------------|-------|
| Nucleotide 495   | A insertion | Allele Frequency | 1.4%  |
| 5 Nucleotide 600 | A/G         | Allele Frequency | A 92% |
|                  |             |                  | G 8%  |

This polymorphism can be detected by digestion with restriction enzyme Ban II

|              |                 |              |                 |
|--------------|-----------------|--------------|-----------------|
| ....AAGCCC.. | Ban II negative | ...GAGCCC... | Ban II positive |
| ....TTCGGG.. |                 | ...CTCGGG..  |                 |

|                   |     |                  |       |
|-------------------|-----|------------------|-------|
| 10 Nucleotide 809 | C/T | Allele Frequency | C 55% |
|                   |     |                  | T 45% |

This polymorphism can be detected by engineered restriction site Psp1406I (AACGTT)

Engineered primer 787-808 **GGGTGAACAAAAGAAGGAACGT** (SEQ ID No. 6) co-operating with the polymorphism C/T to create the Psp1406I (AACGTT) site only if the "T"

15 polymorphism is present in the target sequence.

**CLAIMS**

What is claimed is:

1. A method for diagnosis of one or more single nucleotide polymorphism(s) in NK1R gene in a human, which method comprises determining the sequence of the nucleic acid of the human at one or more positions:
  - 2361 in exon 1 as defined by the position in SEQ ID No. 1;
  - 1371 in the promoter element as defined by the position in SEQ ID No.1;
  - 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
  - 10 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
  - 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;
  - 461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
  - 495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
  - 600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
  - 15 809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;
 and determining the status of the human by reference to polymorphism in NK1R.
2. A method according to claim 1 in which the single nucleotide polymorphism at position 2361 in exon 1 is presence of C and/or T, the single nucleotide polymorphism at position 1371 in the promoter element is presence of A and/or G, the single nucleotide polymorphism at position 271 near exon 3 is presence of G and/or T, the single nucleotide polymorphism at position 272 near exon 3 is presence of A and/or a single base deletion at this position, the single nucleotide polymorphism at position 245 in exon 5 is presence of C and/or a single base deletion at this position, the single nucleotide polymorphism at position 25 461 in the 3' UTR is presence of G and/or C, the single nucleotide polymorphism at position 495 in the 3' UTR is the presence of T and/or a single base insertion of A at this position, single nucleotide polymorphism at position 600 in the 3' UTR is presence of A and/or G, the single nucleotide polymorphism at position 809 in the 3' UTR is presence of C and/or T.
- 30 3. A method as claimed in claim 1 or 2, wherein the region containing the potential polymorphism is amplified by polymerase chain reaction prior to determining the sequence.

4. A method as claimed in any of claims 1 - 3, wherein the presence or absence of the polymorphism is detected by reference to the loss or gain of, optionally engineered, sites recognised by restriction enzymes.
5. A method according to claim 1 or claim 2, in which the sequence is determined by a method selected from ARMS-allele specific amplification, allele specific hybridisation, oligonucleotide ligation assay and restriction fragment length polymorphism (RFLP).
6. A method as claimed in any of the preceding claims for use in assessing the predisposition and/or susceptibility of an individual to diseases mediated by NKIR ligands.
7. A nucleic acid comprising any one of the following polymorphism containing sequences:
  - the nucleic acid sequence of SEQ ID No. 1 with T at position 2361 in exon 1 as defined by the position in SEQ ID No. 1;
  - the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with T at position 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
  - the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with a single base deletion at position 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
  - the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with a single base deletion at position 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;
  - the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with C at position 461 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;
  - the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with A inserted at position 495 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;
  - the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with G at position 600 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;
  - the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with T at position 809 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;
  - the nucleic acid sequence of SEQ ID No. 1 with G at position 1371 in the promoter element as defined by the position in SEQ ID No. 1;



or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms.

8. A diagnostic nucleic acid primer capable of detecting a polymorphism in the NK1R gene at one or more of positions: 2361 in exon 1 as defined by the position SEQ ID No. 1; 1371 in the promoter element as defined by the position SEQ ID No. 1; 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181; 461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181; 495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181; 600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181; 809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181, in the NR1R gene.

15 9. A diagnostic primer as claimed in claim 8 which is an allele specific primer adapted for use in ARMS.

10. An allele-specific oligonucleotide probe capable of detecting a polymorphism in the NK1R gene at one or more of positions: 2361 in exon 1 as defined by the position in SEQ ID No. 1; 1371 in the promoter element as defined by the position in SEQ ID No. 1; 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181; 461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181; 495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181; 600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181; 809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181, in the NR1R gene.

11. A diagnostic kit comprising one or more diagnostic primer(s) as defined in claim 8 or 9 and/or one or more allele-specific oligonucleotide probes(s) as defined in claim 10.

12. An allelic variant of human NK1R polypeptide having a C-terminal deletion of 26 amino acids.

13. A method of treating a human in need of treatment with an NK1R ligand antagonist drug in which the method comprises:

(i) diagnosis of a single nucleotide polymorphism in the NK1R gene in the human, which diagnosis comprises determining the sequence of nucleic acid at one or more of positions: 2361 in exon 1 as defined by the position SEQ ID No. 1;

1371 in the promoter element as defined by the position in SEQ ID No. 1;

10 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

15 600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

and determining the status of the human by reference to polymorphism in the NK1R gene;

and

(ii) administering an effective amount of a NK1R ligand antagonist.

20

14. Use of an NK1R ligand antagonist drug in preparation of a medicament for treating a NK1R ligand mediated disease, particularly asthma, in a human diagnosed as having a single nucleotide polymorphism at one or more of positions:

2361 in exon 1 as defined by the position in SEQ ID No. 1;

25 1371 in the promoter element as defined by the position in SEQ ID No. 1;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

30 495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181.

15. A pharmaceutical pack comprising an NK1R antagonist drug and instructions for administration of the drug to humans diagnostically tested for a single nucleotide polymorphism at one or more positions:

2361 in exon 1 as defined by the position in SEQ ID No. 1;

5 1371 in the promoter element as defined by the position in SEQ ID No. 1;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

461 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

10 495 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

600 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181;

809 in the 3' UTR as defined by the position in EMBL ACCESSION NO. X65181.

16. A computer readable medium having stored thereon a member selected from the group

15 consisting of: a nucleic acid comprising SEQ ID No. 1; a set of nucleic acids wherein at least one of said sequences comprises SEQ ID No. 1; a data set representing a nucleic acid sequence comprising SEQ ID No. 1; a nucleic acid consisting of SEQ ID No. 1; a set of nucleic acids wherein at least one of said sequences consists of the sequence of SEQ ID No. 1; a nucleic acid comprising any part of a sequence selected from the group consisting of: SEQ  
20 ID No. 1, EMBL ACCESSION NO. X 65177, EMBL ACCESSION NO. X 65179, EMBL ACCESSION NO. X 65179 or EMBL ACCESSION NO. X 65181, which part includes at least one of the polymorphisms identified in claim 1.

17. A method for performing sequence identification, said method comprising the steps of  
25 providing a nucleic acid sequence comprising a sequence selected from the group consisting of: the nucleic acid sequence of SEQ ID No. 1 with T at position 2361 in exon 1 as defined by the position in SEQ ID No. 1; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with T at position 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with a single  
30 base deletion at position 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with a single base deletion at position 245 in exon 5 as defined by the position in EMBL ACCESSION NO.

X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with C at position 461 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with A inserted at position 495 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with G at position 600 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with T at position 809 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of SEQ ID No. 1 with G at position 1371 in the promoter element as defined by the position in SEQ ID No. 1; or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms; and comparing said nucleic acid sequence to at least one other nucleic acid or polypeptide sequence to identify identity.

18. A method for performing sequence identification, said method comprising the steps of  
15 providing one or more of the following polymorphism containing nucleic acid sequences: the nucleic acid sequence of SEQ ID No. 1 with T at position 2361 in exon 1 as defined by the position in SEQ ID No. 1; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with T at position 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with a single base  
20 deletion at position 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with a single base deletion at position 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with C at position 461 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the  
25 nucleic acid sequence of EMBL ACCESSION NO. X 65181 with A inserted at position 495 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with G at position 600 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with T at position 809 in the 3'UTR as defined by the  
30 position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of SEQ ID No. 1 with G at position 1371 in the promoter element as defined by the position in SEQ ID No. 1; or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at

least one of the polymorphisms, in a computer readable medium; and comparing said nucleic acid sequence to at least one other nucleic acid or polypeptide sequence to determine identity.

FOR UTILITY/DESIGN  
CIP/PCT NATIONAL/PLANT  
ORIGINAL/SUBSTITUTE/SUPPLEMENTAL  
DECLARATIONS

RULE 63 (37 C.F.R. 1.63)  
DECLARATION AND POWER OF ATTORNEY  
FOR PATENT APPLICATION  
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PM & S  
FORM

Z70365/UST

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the **INVENTION ENTITLED GENETIC POLYMORPHISMS IN THE HUMAN NEUROKININ 2 RECEPTOR GENE AND THEIR USE IN DIAGNOSIS AND TREATMENT OF DISEASES**

the specification of which (CHECK applicable BOX(ES))

X ☐ A. ☐ is attached hereto.  
B. ☐ was filed on \_\_\_\_\_ as U.S. Application No. \_\_\_\_\_  
C. ☒ was filed as PCT International Application No. PCT/CB99/02340 on 20.07.1999  
and (if applicable to U.S. or PCT application) was amended on \_\_\_\_\_

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56. Except as noted below, I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International Application which designated at least one other country than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International Application, filed by me or my assignee disclosing the subject matter claimed in this application and having a filing date (1) before that of the application on which priority is claimed, or (2) if no priority claimed, before the filing date of this application:

PRIOR FOREIGN APPLICATION(S)

| Number    | Country | Day/MONTH/Year Filed | Date first Laid-open or Published | Date Patented or Granted | Priority NOT Claimed |
|-----------|---------|----------------------|-----------------------------------|--------------------------|----------------------|
| 9816192.0 | GB      | 25.07.1998           |                                   |                          |                      |
| 9818280.1 | GB      | 22.08.1998           |                                   |                          |                      |

If more prior foreign applications, X box at bottom and continue on attached page.

Except as noted below, I hereby claim domestic priority benefit under 35 U.S.C. 119(e) or 420 and/or 365(c) of the indicated United States applications listed below and PCT international applications listed above or below and, if this is a continuation-in-part (CIP) application, insofar as the subject matter disclosed and claimed in this application is in addition to that disclosed in such prior applications, I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56 which became available between the filing date of each such prior application and the national or PCT international filing date of this application:

PRIOR U.S. PROVISIONAL, NONPROVISIONAL AND/OR PCT APPLICATION(S)

| Application No. (series code/serial no.) | Day/MONTH/Year Filed | Status                       | Priority NOT Claimed |
|--|----------------------|------------------------------|----------------------|
|  |                      | pending, abandoned, patented |                      |

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

And I hereby appoint Pillsbury Madison & Sutro LLP, Intellectual Property Group, 1100 New York Avenue, N.W., Ninth Floor, East Tower, Washington, D.C. 20005-3918, telephone number (202) 661-3000 (to whom all communications are to be directed), and the below-named persons (of the same address) individually and collectively my attorneys to prosecute the application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent, and I hereby authorize them to delete names/numbers below of persons no longer with their firm and to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/organization to which this case is assigned and by whom I hereby declare that I have consented after full disclosure to be represented unless/until I instruct the above firm and/or a below attorney in writing to the contrary.

|                    |       |                    |       |                      |       |                      |       |
|--------------------|-------|--------------------|-------|----------------------|-------|----------------------|-------|
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| Raymond F. Lippitt | 17519 | Paul E. White, Jr. | 32011 | Stephen C. Glazier   | 31361 | W. Patrick Bengtson  | 32456 |
| G. Lloyd Knight    | 17698 | Glenn J. Perry     | 28458 | Paul F. McQuade      | 31542 | Jack S. Banfika      | 37087 |
| Carl G. Love       | 18781 | Kendrew H. Colton  | 30368 | Ruth N. Morduch      | 31044 | Adam R. Hess         | 41835 |
| Kevin E. Joyce     | 20608 | G. Paul Edgell     | 24239 | Richard H. Zaitlen   | 27248 |                      |       |
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| Peter W. Gowdny    | 25872 | Daniel A. Jakopin  | 32995 | Anita M. Kirkpatrick | 32517 |                      |       |

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*John C. Smith* Date: 1st December 2000

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*Rakesh Anand* Date: 24th November 2000

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| (include Zip Code)  | Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom |             |

FOR ADDITIONAL INVENTORS, "X" box ☒ and proceed on the attached page to list each additional inventor.

☐ See additional foreign priorities on attached page (incorporated herein by reference).

Atty. Dkt. No. PM

(M#)

PAT-116 (10/99)

DECLARATION AND POWER OF ATTORNEY  
(continued)

Z70365/UST

Page 2

ADDITIONAL INVENTORS

(3) INVENTOR'S SIGNATURE: *John*

Date: *24 November 2000*

|   |   |                       |
|---|---|-----------------------|
| First                                     | Middle Initial  | Family Name           |
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| Residence                                 | City  | State/Foreign Country |
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| Post Office Address<br>(include Zip Code) | <i>Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom</i> |                       |

(4) INVENTOR'S SIGNATURE:

Date:

|   |                        |                       |
|---|------------------------|-----------------------|
| First                                     | Middle Initial         | Family Name           |
| Residence                                 | City                   | State/Foreign Country |
| Post Office Address<br>(include Zip Code) | Country of Citizenship |                       |

(5) INVENTOR'S SIGNATURE:

Date:

|   |                        |                       |
|---|------------------------|-----------------------|
| First                                     | Middle Initial         | Family Name           |
| Residence                                 | City                   | State/Foreign Country |
| Post Office Address<br>(include Zip Code) | Country of Citizenship |                       |

(6) INVENTOR'S SIGNATURE:

Date:

|   |                        |                       |
|---|------------------------|-----------------------|
| First                                     | Middle Initial         | Family Name           |
| Residence                                 | City                   | State/Foreign Country |
| Post Office Address<br>(include Zip Code) | Country of Citizenship |                       |

(7) INVENTOR'S SIGNATURE:

Date:

|   |                        |                       |
|---|------------------------|-----------------------|
| First                                     | Middle Initial         | Family Name           |
| Residence                                 | City                   | State/Foreign Country |
| Post Office Address<br>(include Zip Code) | Country of Citizenship |                       |

(8) INVENTOR'S SIGNATURE:

Date:

|   |                        |                       |
|---|------------------------|-----------------------|
| First                                     | Middle Initial         | Family Name           |
| Residence                                 | City                   | State/Foreign Country |
| Post Office Address<br>(include Zip Code) | Country of Citizenship |                       |

(9) INVENTOR'S SIGNATURE:

Date:

|   |                        |                       |
|---|------------------------|-----------------------|
| First                                     | Middle Initial         | Family Name           |
| Residence                                 | City                   | State/Foreign Country |
| Post Office Address<br>(include Zip Code) | Country of Citizenship |                       |

-1-

## SEQUENCE LISTING

&lt;110&gt; Zeneca Limited

5 &lt;120&gt; CHEMICAL COMPOUNDS

&lt;130&gt; LDSG/PHM 70365/WO

&lt;140&gt;

10 &lt;141&gt;

&lt;150&gt; 9816192.0

&lt;151&gt; 1998-07-25

15 &lt;160&gt; 6

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

20 &lt;211&gt; 2547

&lt;212&gt; -DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 1

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30 ctcatcccat cttggttctt ccaaaagggg ttatacggac tggcgagtct ttgacctgcc 360  
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